

**{Exhibit 69}**

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# ORGANIC CHEMISTRY OF NUCLEIC ACIDS

## Part B

Edited by N. K. Kochetkov and E. I. Budovskii

Translated from Russian by Basil Haigh

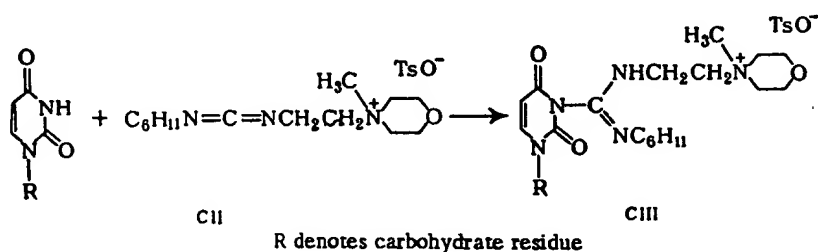
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acrylic anhydride, 1-carbomethoxyethyl- and 1-carboxyethyl-2'-3'-O-isopropylideneinosines respectively were obtained [300]. Treatment of 2',3'-O-isopropylidene-uridine and thymidine with acrylic anhydride yields only the corresponding 5'-O-acryloyl derivatives.

### 3. Interaction with reagents containing C=N bonds

The only known reaction of this type is that between nucleosides and salts of N-cyclohexyl-N'-(methylmorpholinium)-ethylcarbodiimide (CII). Nucleosides containing the  $\text{-CONH-}$  group in the heterocyclic ring take part in the reaction [301, 302]; in the case of uridine, the 3-N-substituted derivative CIII is formed [301-303].



The corresponding reaction has been shown to take place with derivatives of guanosine [301, 302], deoxyguanosine [302], thymidine [302], inosine [304], and pseudouridine [305]; in the last case a mixture of the 3-mono- and the 1,3-disubstituted nucleosides is formed. The reaction proceeds rapidly in a weakly alkaline medium. A study of the effect of pH on the reaction velocity shows that the anion of the nucleoside takes part in the reaction. The reaction velocity decreases in the order: inosine > uridine > guanosine [304] (Table 5.7).

Adducts formed from nucleosides and the carbodiimide CII decompose in a weakly alkaline medium with regeneration of the nucleoside. An exception is the 3-monomonsubstituted derivative of pseudouridine, which is resistant to the action of dilute ammonia [305]. Because of this fact, the specific modification of tRNA at its pseudouridine residues becomes possible, in principle, by treating it with the carbodiimide CII and keeping it in a weakly alkaline medium. However, this suggestion has not yet been verified experimentally.

At pH values below 7, interaction between nucleotides and the carbodiimide CII proceeds at the phosphate group, leading principally to conversion of the nucleoside-2'(3')-phosphates into cyclic phosphates, and of nucleoside-5'-phosphates into oligonucleotides [307]. In the case of phosphodiester, however, no side reaction at the phosphate group is observed, so that the carbodiimide CII can be used successfully to modify polynucleotides.

Single-stranded polynucleotides with no intramolecular hydrogen bonds between the bases (polyuridylic acid, for example), react smoothly with the

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TABLE 5.7. Velocity Constants of Pseudo First-Order Reaction of Nucleosides and Polynucleotides with Tosylate (CII) (0.1 M Tris-HCl Buffer; pH 8.0; 23°C; 0.01 M  $Mg^{++}$ )

Nucleoside or polynucleotide	$k \cdot 10^3, \text{ min}^{-1}$		Nucleoside or polynucleotide	$k \cdot 10^3, \text{ min}^{-1}$	
	0.047 M CII	0.188 M CII		0.047 M CII	0.188 M CII
Uridine	3.4	13.7	Polyuridylic acid	2.2	8.8
Guanosine	2.2	8.8	Valine tRNA from yeast	1.7	1.7
Inosine	9.3	37.2			

carbodiimide CII [304, 308]; the reaction velocity in this case is somewhat lower than for uridine (Table 5.7). Virtually no reaction takes place with double-stranded complexes of polyribonucleotides and DNA [308]. The rate and degree of interaction between the carbodiimide CII and tRNA are strongly dependent upon the reaction conditions [306]. At pH 8 and 30–40°C, complete modification of all reactive nucleoside residues is obtained; in the presence of  $Mg^{++}$  ions and at a lower temperature the degree of modification is slight and is dependent on the concentration of the reagent [304]. In the reaction between the carbodiimide CII and the individual alanine tRNA from yeast, in the presence of magnesium ions, no modification takes place in the area of the sequence pTp $\Psi$ pCpGpApU [309]. Specific partial modification of 5S RNA from *E. coli* has also been carried out by the action of a carbodiimide [361]. Further information regarding the reaction of carbodiimides with nucleotides and tRNA is given elsewhere [362].

Modification of polynucleotides by carbodiimides leads to a substantial change in their susceptibility to nuclease attack. Dinucleotides containing a modified uridine residue are resistant to the action of pancreatic pyrimidyl-RNase [301, 302]. This allows the specific enzymochemical cleavage of RNA at cytidylic acid residues [302, 310, 311], a method which has been used to establish the structure of 5S RNA from *E. coli* (see page 61), and for the preparative synthesis of trinucleotides containing a cytidine residue at the 3'-end. Dinucleotides containing a residue of uridine or pseudouridine, modified by carbodiimide, at the 3'-end are resistant to the action of phosphodiesterases from snake venom and the spleen [305].

Because the course of the reaction with the carbodiimide CII is so strongly dependent on secondary structure, and because of restriction of nuclease action after modification, the reaction with this carbodiimide can be used to identify polynucleotide segments in which separation of the double-stranded complex takes place during partial denaturation of DNA [312]. After treatment of DNA with the carbodiimide CII, followed by treatment with pancreatic DNase and phosphodiesterase from snake venom, long oligonucleotides arising from "defective" segments of the polymer can be isolated.

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